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Resupinate Dimorphy, a novel pollination strategy in two-lipped flowers of *Eplingiella* (Lamiaceae)

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ABSTRACT

This work provides a summary of the typical floral structure of subtribe Hyptidinae (Lamiaceae), in which both style and stamens are declinate within or near the concave anterior corolla lobe. Cross-pollination is facilitated by protandry, acting in conjunction with the explosive release of the stamens and pollen. In contrast, we report that in the three species of the genus *Eplingiella* we found individuals with either resupinate or non-resupinate flowers, which represents a novel floral dimorphism. In these species of *Eplingiella*, the style occupies a position towards the posterior corolla lip and opposes the declinate stamens. Thus, in non-resupinate flowers the pollinating bee receives pollen on its ventral side and makes contact with the style on its dorsal side, whereas in resupinate flowers, the bee receives pollen on its dorsal side, and contacts the style on its ventral side. Both floral morphs seem to be required to achieve cross-pollination. In the two populations studied, each of the two morphs is present and in similar proportions, providing a novel means of promoting cross-pollination and reducing selfing. The situation in *Eplingiella* is compared to some other examples of floral polymorphism, but appears to be a unique pollination strategy, here termed Resupinate Dimorphy.

Keywords: Brazil, Centris, Eplingiella, Hyptidinae, Lamiaceae, resupinate dimorphy, style position

Introduction

The large family Lamiaceae (or Labiatae), to which *Eplingiella* belongs, contains over 7000 species with an almost worldwide distribution. Most species produce nectar, and many are bee-pollinated. The family is notably composed of self-compatible species, and no self-incompatible species have been recorded (Owens & Ubera-Jiménez 1992). Many Lamiaceae, particularly members of the subfamilies Nepetoideae and Lamioideae, are characterized by having zygomorphic, two-lipped flowers, as in *Salvia* and *Lamium*. In these genera, both the stamens and style are ascending and positioned under the upper (posterior) corolla lip, and the chance of self-pollination (at least autonomous autogamy, if not geitonogamy) is reduced by protandry, with the style elongating and the stigma receptive only after the pollen has been shed (Harley *et al.* 2004). In most such species pollination is nototribic, i.e. the pollen is deposited on the dorsal surface of the pollinator. In many *Salvia* species pollen deposition is facilitated by a hinged staminal lever mechanism (Classen-Bockhoff *et al.* 2004). However, in species of the Ocimeae subtribe Hyptidinae, the central anterior corolla-lobe is boat-shaped, concave, laterally

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compressed and with an elastic hinge at its base, such that at early anthesis, the declinate stamens are trapped within it and held under tension by the hinge. This creates an explosive mechanism of pollen release. The slight movement of a visiting pollinator (typically a bee) is sufficient to release the stamens and their pollen explosively, as the hinged lip flicks back, depositing the pollen onto the ventral surface of the insect (Harley 1971; 1976), and the flowers are therefore sternotribic. After the pollen is shed, the stamen filaments curl downwards, and the hitherto short style, which lies slightly above the anterior lip, elongates and the stigma becomes receptive .

The Hyptidinae genus *Eplingiella* consists of three shrubby species up to 3 m tall that are all native to semi-arid areas in NE Brazil (Harley & Pastore 2012; Harley 2014). *Eplingiella brightoniae*, a multicaulous, fastigiate shrub to 1.5m, is restricted to the municipalities of Umburanas and Sento Sé, in very remote, dry montane habitats (*campos rupestres*) in Northern Bahia. *E. cuniloides* with a similar habit to the previous species, is known from a single area in the Morro do Chapéu municipality of Central Bahia, over sands and shallow rocky habitats. *E. fruticosa* is a much branched shrub to 3 m tall, which occurs widely in semi-arid caatinga vegetation and in coastal sands in Bahia, and in other states in the semi-arid Northeast of Brazil (Harley 2014).

When dissecting flowers in order to describe *Eplingiella brightoniae* (Harley 2014), we noted that the flowers were often, but not invariably resupinate. This prompted more detailed studies so as to investigate to what extend this situation might affect the pollination process and to see whether the phenomenon was exclusive to this species.

Materials and methods

Previous studies of the flowers of *Eplingiella brightoniae* Harley, *E. cuniloides* (Epling) Harley & J.F.B. Pastore and *E. fruticosa* (Salzm. ex Benth.) Harley & J.F.B. Pastore in the herbaria of the Royal Botanic Gardens, Kew (K), and at the Universidade Estadual de Feira de Santana (HUEFS), revealed that plants in each of these three species had one of two types of flower, resupinate or non-resupinate. A detailed study of the inflorescence of *E. brightoniae* was carried out, with the aid of a high-powered binocular microscope, to see whether the position of the bracteoles below the flower might indicate where the axis was twisted, to produce resupination.

Complementary fieldwork was carried out in Bahia state, Brazil, in April 2015, to assess the floral morphs distribution in individuals of *E. cuniloides* (Morro do Chapéu Municipality, Cachoeira do Ferro Doido, 11°37'39.7"S, 41°00'02.4"W, 900 m elev., 6-7th) and *E. brightoniae* (Sento Sé Municipality, Road from Rio Murim to Sento Sé, 10°19'15.3"S, 41°23'41.6"W, 983 m elev., 8–9th).

Both of these areas have a large population with a sufficient number of individuals to carry out a survey of the frequency of the two floral morphs. At each site 100 individuals were selected at random and scored for whether they had resupinate or non-resupinate flowers. Additionally, flowers were photographed, and further studies in the herbaria were made for the two species. The bee visitors were collected, and identified by Dr Favizia de Oliveira, and deposited in the BIOSIS Laboratory, Biology Institute, Universidade Federal da Bahia (UFBA).

For *E. fruticosa*, we were unable to find a suitable population with enough individuals for a survey of morph frequencies to be undertaken. However, a cursory examination of a range of individuals of this species in the field, in April 2015 and April 2016, evinced that both resupinate and non-resupinate individuals were present.

Results and discussion

Observations in the field, combined with herbarium studies at K and HUEFS, confirmed that in the three species of *Eplingiella*, the basic floral structure is, with few exceptions, very similar to that found in other Hyptidinae. In subtribe Hyptidinae, flowers at the onset of anthesis have the stamens/anthers held under tension inside the boat-shaped central anterior petal lobe (Fig. 1A), and they are released explosively when visited by a potential pollinator (Fig. 1B). The flowers show marked protandry and the initially short style which lies along the anterior corolla lip subsequently elongates and the stigma becomes receptive. In such flowers, pollen is likely to be deposited on, and picked up by the stigma from the ventral surface of the bee, such that pollination is sternotribic. However, in the three species comprising the genus *Eplingiella* the flowers differed in two important respects from this structure. Firstly, in all flowers the style was no longer found above the declinate stamens but rather more upright and closer to the posterior lip of the corolla and thus occupied a more central position. Secondly, in the populations we studied, ca. half the plants had flowers as described above, with the stamens held within the anterior corolla lobe in a ventral position so that pollen deposition on the vector was sternotribic, whilst in the remaining half of the population, the plants showed resupinate (inverted) flowers so that the stamens were located above the style/stigma and consequently pollen deposition was nototribic. In both cases, the four stamens were held within the boat-shaped median lobe of the anterior corolla-lip and showed a similar explosive mechanism of pollen release (Figs. 1C-G, 2A-F).

Resupination of the flower by torsion of the pedicel has been demonstrated in other Lamiaceae, e.g. *Salvia jurisicii* (Schmucker 1929; Turrill 1929). However, with a pedicel of often less than 1 mm in *E. brightoniae*, we have so far been unable to confirm such torsion. As in other Hyptidinae, the structure of the cyme is dichasial, and in this species the cymes are 3 – 5 flowered. These are sessile, or with a much

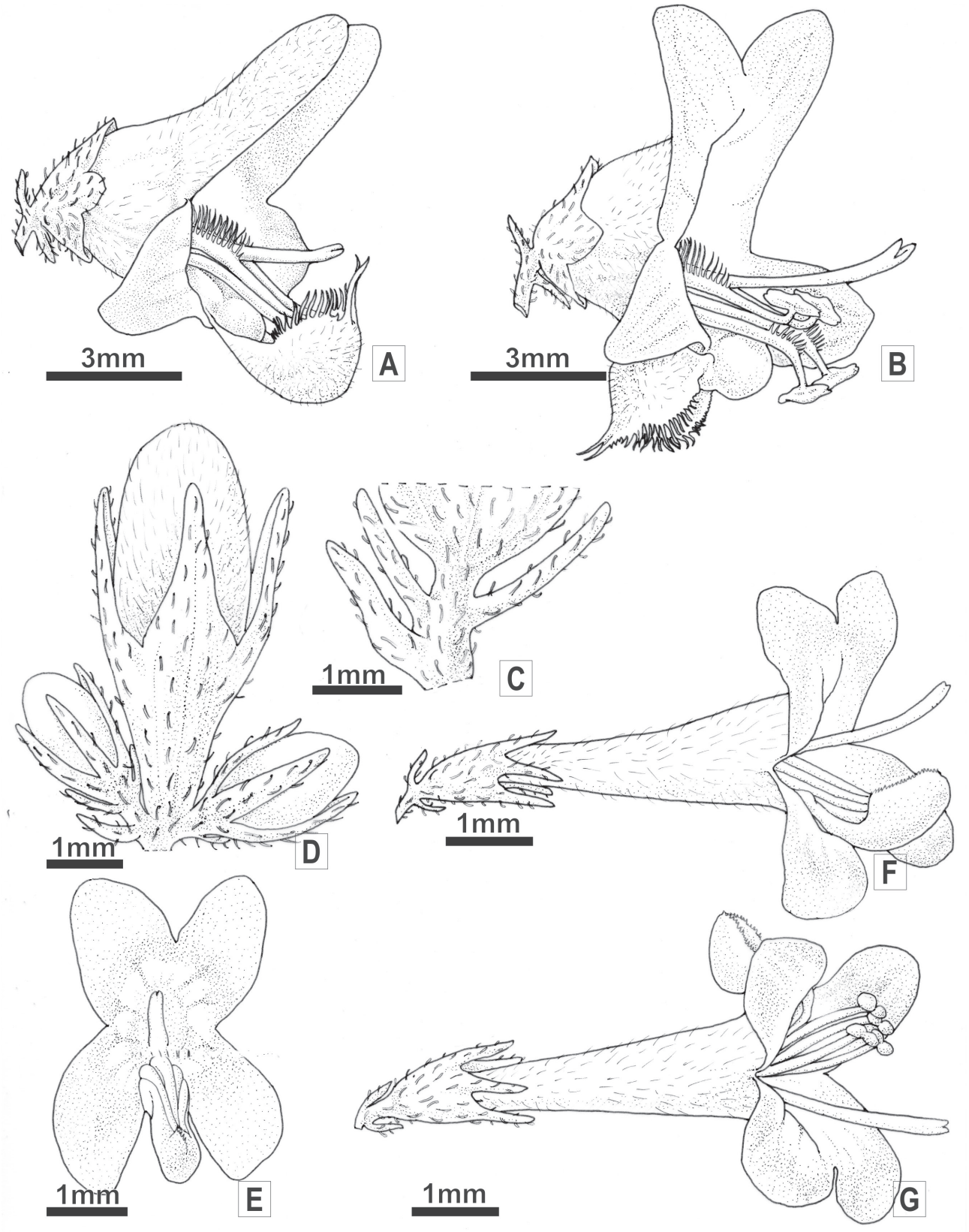

Figure 1. A-B. *Eriope crassipes* (based on Harley 1976: Tab. 3751 C, D) - A. untriggered flower, B. flower triggered and stamens with dehisced anthers curving down, away from the style. C-D. *Eplingiella brightoniae* – C. cyme detail, base of young flower, with bracteoles; D. cyme detail, triad with bracteoles. E. *E. fruticosa* – face view of untriggered flower, showing stamens held in anterior median corolla lobe. F-G. *E. brightoniae* – F. non-resupinate untriggered flower, side view; G. resupinate flower , side view, with triggered corolla lobe reflexed.

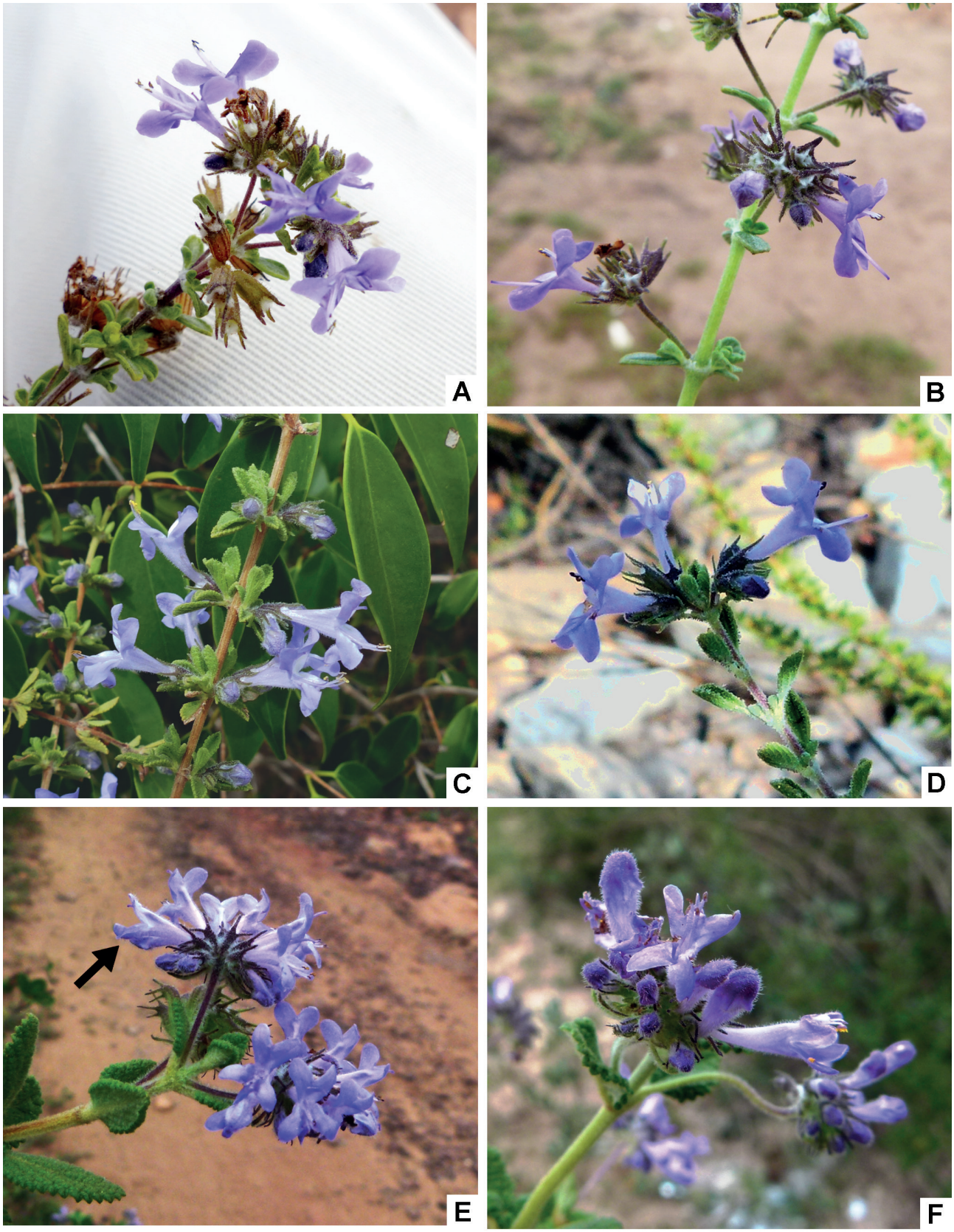

Figure 2. A-B. *Eplingiella cuniloides* – A. non-resupinate flower; B. resupinate flower. C-D. *E. brightoniae* - C. non-resupinate flower; D. resupinate flower. E-F. *E. fruticosa* - E. non-resupinate flower (arrow indicates an untriggered flower, with adaxial lobe not yet reflexed); F. resupinate flower.

foreshortened peduncle up to 2 mm long, subtended by a small bract and two lateral bracteoles at the base of the flower (Fig. 1C-D). Nevertheless, it seems probable that resupination was due to a twisting of the short pedicel, although this could not be seen.

Scoring 100 individuals in the populations of *Eplingiella cuniloides* and *E. brightoniae*, demonstrated that every plant possessed either non-resupinate flowers or resupinate flowers and that the different morphs occurred in almost equal proportions in each population: *E. cuniloides* 47 non-resupinate, 53 resupinate; *E. brightoniae* 44 non-resupinate and 56 resupinate.

In *Eplingiella cuniloides* our observations were restricted to the afternoon, when there was a long period of sunshine and we observed bees visiting the flowers. Two specimens were captured, and were identified as a male and a female *Centris caxiensis*. The size and position of the bees on the flower were adequate to effect pollination, and pollen grains present were sparsely dispersed over the body, and not in scopae on the hind legs. In general, *Centris* bees collect floral oils, which are not present in Lamiaceae, so we assume that the visits were primarily to take nectar. Moura (2008) records a visit by *Acamptopoeum prinii* Andrenidae to the flowers of "*Hyptis fruticosa*" (until 2012 the accepted name for *Eplingiella fruticosa*). This is a smaller bee than *Centris caxiensis*, and is primarily a collector of pollen.

The floral dimorphy involving individuals with non-resupinate and resupinate flowers observed in these three species of *Eplingiella* seems to function as an adaptation to promote cross-pollination. To achieve pollination, the insect bearing pollen needs to visit the other morph. For example, the insect which has visited a non-resupinate flower, with pollen on its ventral surface, on alighting on a resupinate flower, will pass pollen onto the stigma, which is below, and receive pollen, this time on its dorsal side. Whichever morph the insect next visits, it can only pass pollen onto the stigma of a flower of the opposite morph, thus ensuring cross-pollination, as each individual plant possesses only one type of flower. With this regime in operation, protandry would appear to be no longer necessary, and it will be interesting to examine a range of individuals to see whether there has been any change in the timing of style elongation and maturation of the stigma, in relation to the release of pollen. Photographs taken on site, and preliminary field observations suggest that protandry still occurs, but when observations were made there were very few flowers remaining untriggered.

This floral dimorphy, with non-resupinate and resupinate flowered plants within populations, seems to be unique to *Eplingiella*. Resupination is especially well known in orchids, but a number of families of the Lamiales also have some resupinate-flowered species (Clark & Zimmer 2003; Clark *et al*. 2006; Endress 2012). In the Lamiaceae, *Salvia jurisicii*, from Balkan Peninsula has resupinate flowers, where the pedicels twist through 180° as the flowers open (Schmucker 1929; Turrill 1929; Hill 1939; Classen-Bockhoff *et al*. 2004). Two Mexican species of *Salvia*, *S. gravida* and *S. vazquezii* also have resupinate flowers. Here the inflorescences are nodding, and resupination returns the flowers to the normal position with the posterior corolla lip uppermost (Iltis *et al*. 2012). In the Lamiaceae tribe Hyptidinae, the genus, *Hypenia*, has a small group of six red-flowered species, all of which have resupinate flowers. These have relatively large corollas, which are reported to be pollinated exclusively by hummingbirds (Atkinson 1998). However, in all of these cases, resupinate flowers is a feature of the species and is present in all of the individuals, and we know of no other examples of resupinate - non-resupinate flowers occurring as a floral dimorphy, as in these *Eplingiella* species.

Floral polymorphisms (mostly dimorphisms) promoting cross-pollination have been the object of many studies. The commonest situation is floral heterostyly with di- or more rarely tri-stylous with the species having dimorphic style and anther lengths. Additionally, heterostylous species usually possess a heteromorphic incompatibility system, although in *Lithodora* and *Glandora*, two heterostylous genera of Boraginaceae, both self-compatibility and self-incompatibility, along with different degrees of inter-morph compatibility, have been described (Ferrero *et al*. 2012). Here it seems that stylar polymorphism and incompatibility have evolved independently. In the genus *Salvia*, one species, *S. brandegeei*, from North America, shows stylar dimorphy but with no other features of classic heterostyly (Barrett *et al*. 2000). In a series of papers, Jesson & Barrett (2002; 2003) describe the occurrence of enantiostyly in several dialypetalous species of Haemodoraceae and in Pontederiaceae, where there are two morphs, which differ by the lateral deflection of the style within the flower, to the left or to the right, often accompanied by a reciprocal switch to the other side by the stamens (reciprocal enantiostyly). They distinguish between dimorphisms where the two floral morphs occur on the same plant (monomorphic enantiostyly), and where both morphs occur on different plants (dimorphic enantiostyly).

The dimorphic system closest to that found in *Eplingiella* is "inversostyly", as described in *Hemimeris racemosa* (Scrophulariaceae) by Pauw (2005). In this species the style is turned upward in some individuals and downward in others. But there is no resupination involved. In *Alpinia* (Zingiberaceae) a similar mechanism occurs, but combined with heterodichogamy, where the style moves during anthesis either upwards or downwards (termed flexistyly by Li *et al*. 2001).

However, in *Eplingiella* both morphs are structurally identical with a stylar deflection that is the same for both morphs, and only the presence or absence of resupination provides the dimorphism. Thus, we have chosen the term Resupinate Dimorphy, to define the dimorphic flowers of *Eplingiella*, in which resupination is the key. As far as we are aware, these three species of *Eplingiella* are the first case of this novel form of floral dimorphy, providing yet another

useful diagnostic character for the genus. The effect of this novel mechanism on pollinators and their behaviour at the flowers, possible differences on the dichogamy (occurrence of protandry or not), and more general morph ratios and distribution remain to be studied.

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